Pediatr Neonatol 2009;50(6):247–254

REVIEW ARTICLE

The Epithelial Gatekeeper Against Food Allergy

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Received: May 14, 2009
Revised: Jul 27, 2009
Accepted: Jul 29, 2009

KEY WORDS:
allergen uptake;
commensal bacteria;
epithelial permeability;
food allergy;
IgE/CD23;
intestinal barrier function

The rapid rise of allergic disorders in developed countries has been attributed to the hygiene hypothesis, implicating that increased environmental sanitation in early childhood may be associated with higher incidence of hypersensitivity. Intestinal epithelial barriers play a crucial role in the maintenance of gut homeostasis by limiting penetration of luminal bacteria and dietary allergens, yet allowing antigen sampling via the follicle-associated epithelium for generation of tolerance. However, this intricate balance is upset in allergic intestines, whereby luminal proteins with antigenic properties gain access to the subepithelial compartment and stimulate mast cell degranulation. Recent studies demonstrated that food allergens were protected from lysosomal degradation, and were transported in large quantities across the epithelium by binding to cell surface IgE/CD23 (FcεRII) that prevented the antigenic protein from lysosomal degradation in enterocytes. IL-4 (a Th2-type cytokine) not only increased production of IgE from B cells, but also upregulated the expression of CD23 on intestinal epithelial cells. Further studies indicated that CD23 was responsible for the bidirectional transport of IgE across epithelium. The presence of IgE/CD23 opens a gate for intact dietary allergens to transcytose across the epithelial cells, and thus foments the mast cell-dependent anaphylactic responses. The understanding of the molecular mechanism responsible for epithelial barrier defects may be helpful in designing novel therapies to treat food allergy and other allergic diseases.

1. Clinical Prevalence of Food Allergies

Increasing prevalence of allergic diseases, such as asthma, hay fever, eczema and food allergies, has been observed in recent decades. The number of visits due to food-induced anaphylaxis is growing in hospital emergency departments and has significantly increased the burden on medical systems.[1,2] Food allergy is reported in 6–10% of the pediatric population, and is more frequent in children than adults.[3,4] The most common allergens include cow's milk, eggs, peanuts, and seafood. Cow's milk allergy occurs in 2–3% of the infant population, and is mostly diagnosed within the first month of life. Egg allergy is seen in 1–2% and peanut allergy in 0.3% of children. Although some types of food allergy, e.g. cow's milk, remit spontaneously during the first few years of life, they are often associated with later development of respiratory and skin allergic manifestations.[3,4]

2. Hygiene Hypothesis

The rapid rise of allergic disorders in developed countries has led to the postulation that lower incidence of microbial infection in early childhood—due to increased environmental sanitation—may be associated with higher incidence of hypersensitivity.[5] This is termed the hygiene hypothesis. This hypothesis was originally formed in 1989 by Strachan,

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an epidemiologist, who observed an inverse correlation between family size and the occurrence of atopic diseases.[6] Speculations were formed that smaller household size indicated less cross-infection between family members/siblings—this could be related to the development of allergic disorders in individuals. This phenomenon was coined the "post-industrial revolution epidemic". In the following years, allergists and immunologists further broadened the concept that reduced exposure to microorganisms and microbial products, and increased inoculation of antibiotics and vaccines at a young age may predispose individuals to allergic diseases.[6,7]

3. Oral Tolerance and Allergy

Oral tolerance has been defined as a systemic immune unresponsiveness to a specific antigen which was previously orally administered. Studies in experimental models show that the dose and frequency of antigen feeding influences the course of tolerance acquisition.[8,9] The putative concept is that feeding a high dosage of antigen results in clonal deletion or anergy of specific T cell clones that involve Fas/FasL-dependent apoptosis, whereas feeding a low dosage of antigen favors the pathway of active suppression following the induction of regulatory T (Treg) cells in the Peyer's patches (PP). However, it should be kept in mind that the two forms of tolerance are not mutually exclusive but are likely to be overlapped in physiological situations.

PPs are specialized lymphoid follicles in the gut that are implicated as a crucial site for induction of oral tolerance. Luminal antigens are readily taken up by follicle-associated epithelium (mainly by M cells) on PP and delivered to the underlying dendritic cells and lymphocytes for trafficking to mesenteric lymph nodes (MLN).[10,11] Previous studies demonstrated that lymphotoxin (LT)α knock-out mice, or mice treated *in utero* with LTβ receptor-blockade, are devoid of PP and MLN, and fail to develop oral tolerance.[12,13] However, mesenteric lymph nodes have been suggested to be responsible for the induction of high-dose oral tolerance in the absence of PP. An elegant study showed that by treating pregnant LTα knock-out mice with an agonist anti-LTβR monoclonal antibody, their progeny developed MLN but without PP and readily acquired systemic tolerance to the orally administered protein.[14,15]

Following antigen feeding, the presence of antigen-specific CD4+CD25+Treg cells and secretion of suppressive cytokines, e.g. transforming growth factor (TGF) β, are found in the PP compartment.[16–19] The involvement of TGFβ in oral tolerance induction was first suggested in studies using oral feeding of myelin basic protein to suppress experimental autoimmune encephalomyelitis.[20] Neutralizing antibody to TGFβ inhibited the bystander suppression of immune responses conferred by tolerized T cells *in vitro*.[20] Further evidence indicated that TGFβ played a complementary role in facilitating the induction of low dose antigen-induced oral tolerance by CD4+CD25+Treg cells and was crucial for the maintenance of established tolerance.[21,22] Moreover, oral administration of TGFβ along with antigen augments the reduction of antigen-specific immunoglobulin levels, T-cell reactivity, and immediate skin hypersensitivity compared with antigen feeding alone.[22]

Treg cells mediate active suppression and are primary effector cells involved in the generation of oral tolerance. Oral administration of ovalbumin to ovalbumin TCR transgenic mice resulted in a relative increase in the population of CD4+CD25+Tregs expressing CTLA-1. Adoptive transfer of these CD4+CD25+Tregs from antigen-fed ovalbumin TCR transgenic mice suppressed the delayed type hypersensitivity responses in recipient mice, suggesting that this particular cell type is responsible for oral tolerance induction to specific antigens.[23] Recent data indicated that a dendritic cell subset in the lamina propria characterized by the expression of CD103 induced naive T cells to differentiate into CD4+CD25+Foxp3+Treg cells, via a TGFβ and retinoic acid-dependent mechanism.[24,25]

Dysregulation of various functions in PP may promote allergic sensitization. Studies using milk allergic mice have shown that enhanced transfer of aggregates through PP or abnormal interaction between lymphocytes and dendritic cells in PP favors the development of food allergy.[18,26,27] Orally administered aggregated proteins were redirected to uptake through PP which promoted a significant Th2 response and triggered allergic sensitization in a cow's milk-allergic mouse model.[27] Interestingly, subsequent anaphylaxis was only elicited by orally given soluble, but not aggregated, forms of allergen. The findings suggested that the transport of soluble protein via villous epithelial cells was the main pathway for anaphylactic responses.[27]

In healthy individuals, the colonic lumen hosts over 100 trillion commensal bacteria forming the enteric microfloral population, and is established after birth.[28] Enteric bacteria are involved in numerous protective and metabolic functions of the gut, such as preventing colonization of pathogens, degrading non-digestible dietary substances, producing short chain fatty acids and vitamins, and shaping the mucosal immunity.[28–31] The intestinal IgA production is profoundly affected by the colonization of commensal microflora, as shown by the low levels of IgA in germ-free animals, which is corrected after

inoculation of luminal bacteria.[32] Luminal antigen sampling by PP induces class switch recombination of IgM-positive B cells to IgA. The classing switching of IgA is partially dependent on presence of TGFβ and production of retinoic acid by intestinal dendritic cells.[33,34] These IgA-committed B cells drain to the mesenteric lymph nodes, subsequently enter the thoracic duct and bloodstream, and finally home back to the intestinal mucosa. The secretory IgA (sIgA) produced by these lamina propria plasma cells is transported across the intestinal epithelium via the polymeric immunoglobulin receptor into the lumen.[35] Enteric bacteria are coated with sIgA.[36] Recent studies showed that luminal sIgA selectively adhered to M cells in the mouse and human intestinal PP via a novel IgA receptor—this also mediated translocation of bacteria and antigenic products to the underlying dendritic cells.[37,38] The bacteria transported by the sIgA across M cells further induces naïve B cells to differentiate into IgA-committed plasma cells.[39] The roundtrip, bidirectional transport of sIgA and sIgA-mediated bacterial coatings have been implicated in the mechanism of antigen neutralization that curtails bacterial overgrowth.[40,41]

Recent evidence indicated that gut bacterial flora is required for the induction of oral tolerance. In contrast to conventionally-raised animals, germ-free mice do not generate immune tolerance against fed antigen.[42,43] Mice given oral antibiotics during infancy showed an increase in serum levels of IgG1 and IgE, and decreased IgG2a levels that are associated with enhanced IL-4 secretion in stimulated spleen cells, suggesting a Th2-polarized immune response.[44] The absence of T cells in PP under germ-free conditions was implicated in the mechanism of failure of tolerance induction.[45]

4. Immunological Basis of Food Allergy

Food allergy is characterized by sensitization to specific dietary antigens and local inflammation at the encountering site of allergens. The onset of sensitization involves the combination of a number of factors including genetic traits, allergen exposure, intestinal microflora, and environmental stimuli. Recent evidence has suggested that psychological stress also predisposes animals to sensitization against orally given antigens which otherwise induced tolerance.[46]

The sensitization phase of allergy is characterized by the production of IgE and Th2-type cytokine (IL-4, IL-5, and IL-13) responses. Elevated production of IL-4 by mononuclear cells has been demonstrated in the blood and intestinal mucosa of atopic individuals.[47,48] IL-4 induces germ line ε transcript for isotype class switching in B cells and promotes B cell proliferation to increase the synthesis of antigen-specific IgE.[49] In addition to its presence in serum, elevated levels of IgE are detected in the intestinal fluid in food-allergic patients, suggesting that IgE is also secreted into the gut lumen.[50,51] In parasite-infected rats with stimulated IgE production, the concentration of IgE in the intestinal fluid was greater than that in the serum or mesenteric lymph.[52] A previous study demonstrated that radiolabeled IgE (but not IgG) was translocated from the serum into the gut wall and lumen after infusion of rats with IL-4.[53]

IgE binding to the high affinity FcεRI on the surface of mast cells represents the hallmark of allergy. Cross-linking of IgE by specific antigen induces mast cell degranulation and release of mediators, thereby causing anaphylactic responses.[54] Anaphylactic reactions in food allergy are associated with enhanced epithelial ionic transport with passive out flux of water—this is responsible for clinical diarrheal symptoms.[55,56] Mast cell mediators, e.g. histamine and prostaglandin, are involved in the stimulation of epithelial ion secretion.[57,58] Histamine evokes chloride secretion in epithelial cell monolayers through H1-receptors on intestinal epithelial cells, causing elevation in cytosolic calcium level and protein kinase C activation.[59,60] Prostaglandins have been shown to increase the secretory response of intestinal epithelial monolayers directly via cAMP upregulation, or to serve as a mediation step for ion secretion induced by several cytokines and mediators, such as TNFα, serotonin, leukotrienes, and proteases.[61–63]

5. Epithelial Barrier Defects in Intestinal Allergy

It is generally accepted that intestinal anaphylactic reactions are caused by biological mediators released from mucosal mast cells after antigen cross-linking of IgE bound to the cell surface. However, macromolecular food antigens must first cross the intestinal epithelial barrier before gaining access to mast cells in the subepithelial compartment. Abnormal antigen passage through PP has been suggested as one of the mechanisms responsible for the lack of tolerance.[18,26,27] In comparison to the limited exposing region of PPs, villous epithelium constitutes a relatively large surface area and plays a more important role in responsible for the breach of barrier in intestinal allergy.

Physiologically, most dietary proteins are digested by luminal gastric and pancreatic proteases as well as integral brush border enzymes. They are converted to small peptides and amino acids, which are then absorbed by enterocytes via electrogenic or

sodium-dependent transporters. Although a small amount of intact protein is endocytosed by intestinal epithelial cells in normal conditions, most of it is sorted into lysosomal compartments for degradation and therefore, transcytosis of whole proteins are prevented.[64–66] In other words, the transcellular route across epithelial cells contributes partially to exclusion of luminal proteins with antigenic properties. The intestinal epithelial cells are joined at their apical side by tight junctions.[67,68] The transmembranous junctional proteins, e.g. claudins, occludin or junction-associated molecules, are linked to intracellular zonula occludens which are bridges to cytoskeletal actin and myosin filaments. The tight junctional complexes are the rate-limiting step for paracellular diffusion, and only permit small molecules lesser than 500 daltons to cross between cells. Focal disorganization of tight junctional proteins or circumferential contraction of the perijunctional actomyosin ring may increase paracellular permeability.[69–73]

The place in which route food allergens cross the gut epithelium has been extensively explored in the last decade. In rodent models, it was observed that addition of antigen from the luminal or serosal side of the allergic intestine induces strong ion secretory responses, with different timeframes. Antigen challenge to the serosal side of the intestine induces an immediate increase (approximately 30 seconds) in ion secretion, whereas luminal addition of antigen results in a lag phase (approximately 3 minutes) before the occurrence of the secretory response, mediated by mast cell activation.[57] The lag phase of the anaphylactic response after luminal challenge appears to reflect the time for antigen transport across the intestinal epithelial cells to activate mast cells in the lamina propria.[57]

In contrast to protein degradation in normal intestinal epithelial cells, enhanced rates of transcytosis of intact proteins was documented in allergic animals.[74,75] Previous studies modeling luminal allergen challenge demonstrated that increased antigen uptake was observed within the endosomal compartment of jejunal enterocytes in sensitized rats before mast cell activation.[74,76,77] Studies using sensitized Ws/Ws rats (mast cell-deficiency due to mutation of the c-kit gene) and mast cell stabilizing agents provided evidence that mast cell activation does not play a role in the mechanism of heightened apical-to-basolateral transcellular transport of antigen at this timepoint.[76] The uptake of antigen appeared to be specific and the transport pathway was exclusively transcellular within the first 2 minutes post-challenge. This period of specific transcellular antigen transport before mast cell activation was termed phase I.[76,77] The period following mast cell activation by epithelial ion secretory response was termed phase II (Figure 1). During phase II, antigens were visualized not only inside endosomes but also within the tight junctions and paracellular

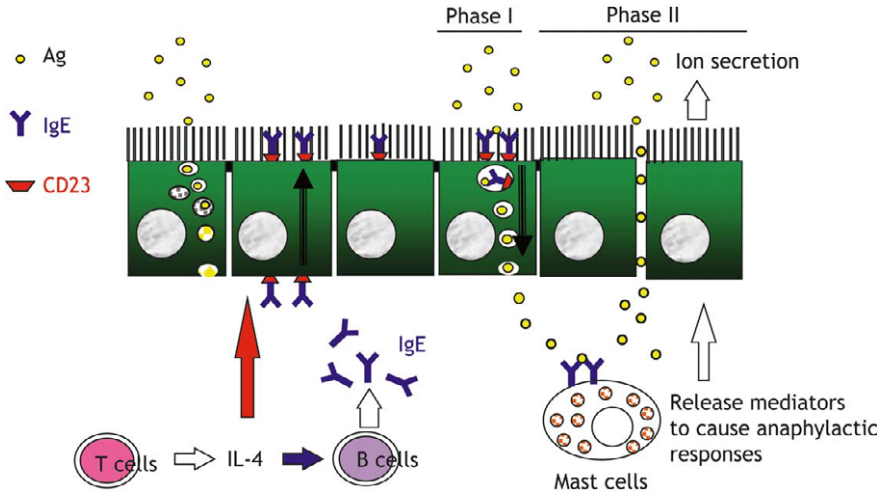

Figure 1 Intestinal epithelial barrier defects in food allergy. Physiologically, most dietary proteins are digested to small peptides and amino acids before being absorbed into enterocytes. A very small percentage of intact proteins may be endocytosed into epithelial cells but are degraded by lysozymes and lose their antigenic properties. In food allergy, IL-4 (a Th2-type cytokine) induces B cells to produce large amounts of IgE to be secreted into serum and gut lumen, or surface-bound onto mast cells. IL-4 also upregulates the expression of low affinity IgE receptor, CD23 (FcεRII), on intestinal epithelial cells. Following exposure to dietary allergens, enhanced transepithelial antigen transport is mediated by IgE/CD23 prior to mast cell activation during phase I. Transcytosed allergens reach the subepithelial lamina propria and cause IgE cross-linking on mast cells, resulting in cell degranulation and anaphylactic responses. The release of mast cell mediators, such as histamine, prostaglandin and proteases, are known to induce epithelial ion secretion and increased paracellular epithelial permeability in phase II.

regions between enterocytes in allergic rats.[76,78] The electrical conductance (measurement of ionic permeability through the paracellular pathway) in intestinal tissues of allergic rats was comparable to those of non-sensitized control animals during phase I. This suggests that gut paracellular permeability was not modified in response to sensitization *per se*. Moreover, a gradual time-dependent increase in tissue conductance corresponds to the phenomenon of enhanced paracellular antigen transport in allergic rats during phase II. The abnormal paracellular epithelial permeability in phase II was absent in allergic mast cell-deficient Ws/Ws rats, suggesting a crucial role of mast cell activation in the induction of tight junction opening and increase of paracellular influx that was not antigen-specific.

Allergic animal models have shown that enhanced transepithelial antigen transport prior to mast cell activation is specific for the allergen to which the rodents are sensitized, suggesting an immunoglobulin recognition mechanism at the level of the epithelium.[74,75] A low affinity IgE receptor, CD23/FcεRII, was previously known for its role in regulating IgE synthesis in B cells and promoting B cell proliferation.[79–81] The expression of CD23 was also found in small intestinal epithelial cells in normal and food allergic humans and rodents, as well as in bronchial epithelial cells in asthmatic patients.[75,77,82] Studies in sensitized rat intestines have demonstrated the translocations of CD23 from cell surface to the membrane of allergen-containing endosomes, confirming the internal-ization of CD23 protein upon luminal antigen challenge.[75] Further studies using genetically mutant mouse models provided evidence for the role of IgE/CD23 in mediating enhanced transepithelial antigen transport in allergy.[77,78] The phenomenon of augmented antigen uptake in allergic enterocytes was completely absent in sensitized CD23$^{-/-}$ mice and IL-4$^{-/-}$ mice. Moreover, the increased transepithelial antigen uptake in allergic wild type mice was inhibited luminally with neutralizing anti-CD23 antibodies.[77,78] Passive sensitization of naive mice by injecting immune serum from allergic mice restored the allergic response, but not if IgE was first depleted from serum, confirming the crucial role of IgE in antigen uptake. In addition, IL-4 increased the expression of CD23 transcript and protein levels in murine intestinal epithelial cell cultures, as well as allergic mouse enterocytes.[77] Overall, enhanced transepithelial antigen transport is mediated by IgE/CD23 and regulated by IL-4 (Figure 1).

Recent studies indicated that various isoforms of CD23 mediated bidirectional transport of IgE across the epithelium in allergic murine and human intestines. DNA sequencing revealed the presence of classical and alternative CD23*b* transcripts lacking exon 5 (*b*Δ5) or 6 (*b*Δ6) in mouse enterocytes, all of which were translated into functional IgE receptors with distinct endocytic properties.[78,83] Mouse intestinal epithelial CD23*b*Δ5 transcripts mediated apical to basolateral transport of free IgE, whereas classical CD23*b* transcripts displayed higher efficiency in the transcytosis of IgE/allergen complexes.[78,83] Studies using primary human intestinal epithelial cells and transformed cell lines have also shown that CD23 transports IgE in both the mucosal-to-serosal and serosal-to-mucosal directions.[84] Both CD23 isoforms *a* and *b* transfected into human intestinal epithelial cells transcytosed bidirectionally by IgE. However, CD23*a* transport IgE/antigen complex was faster than CD23*b* in the apical-to-basolateral direction.[85] Despite the controversy over which isoform is expressed in human enterocytes,[84,85] IgE/CD23 is responsible for enhanced transepithelial antigen uptake that results in mast cell activation. It is noteworthy that food allergens transported *via* the intestinal epithelial IgE/CD23 are protected from lysosomal degradation, and therefore, intact antigenic forms of the proteins are preserved.[86] Taken together, these findings suggest that epithelial CD23 is involved in the bidirectional transport of IgE that accounts for the high luminal concentration of IgE in allergic situations and the enhanced uptake of IgE-bound antigen complexes leading to anaphylactic responses.

6. Novel Therapeutic Interventions

Currently dietary exclusion is still the most effective measure for the prevention of allergic sensitization and anaphylactic responses in high-risk children and adults. Novel immunotherapy by delivering allergen via subcutaneous or sublingual routes have shown some degree of symptom alleviation in experimental models and human studies, yet large scale blinded, placebo-controlled trials are needed to confirm the effects for long-term desensitization.[87] The use of anti-IgE antibody increased the threshold of sensitivity to allergens in a double-blind, randomized, dose-ranging trials for peanut allergic patients.[88] Moreover, targeting CD23 with monoclonal antibody has been shown to decrease total serum IgE levels in approximately 75% of patients in phase I clinical trial of allergic asthma and was proposed as candidate therapy for treating allergic diseases for subgroups of patients.[89] Additional information is needed to develop safe and effective treatments for food allergy. The understanding of the molecular mechanism underlying epithelial barrier defects and induction of allergy may shed light to the advance in clinical management of atopic disorders.

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